Jurnal Teknologi Laboratorium
Vol.9, No.2, 2020, pp. 186 – 191
ISSN 2580-0191(Online), ISSN 2338 – 5634(Print)
DOI: 10.2938/teknolabjournal.v9i2.235
Journal homepage: https://www.teknolabjournal.com/index.php/Jtl/index

Original Research

Identification of molecular markers for type 2 Diabetes mellitus in Sidoarjo, Indonesia

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HIGHLIGHTS

Based on our study we found molecular markers to determine the likelihood of a person suffering from type 2 diabetes using the PCR-RAPD method

ARTICLE INFO

Article history

Received Date: Aug 17th, 2020
Revised Date: Nov 02nd, 2020
Accepted Date: Nov 28th, 2020

Keywords:
Diabetes mellitus
Genetic disorder
PCR-RAPD
Polymorphism
Molecular Marker

ABSTRACT

T2DM can be triggered by two collaborating factors, namely genetics and the environment. This study aimed to identify genetic markers that can be used to detect the possibility of a person having T2D using the random amplified polymorphism DNA (RAPD) method. The study was carried out cross-sectional and involved 60 samples consisting of 30 positive T2D samples and 30 negative samples T2D. The primer used for PCR-RAPD was D20 (5'-ACCCGGTCAC-3'). The PCR-RAPD results were then analyzed using the scoring method and analyzed using the non-parametric Chi-Square test (cl: 95%). Among T2D, 576 bp band were confirmed to be markers in the patients.

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1. INTRODUCTION

Diabetes mellitus (DM) sufferers continue to increase. 463 million people suffer diabetes mellitus and it is estimated to increase to 578 million in 2030 and 700 million in 2045. Indonesia is the seventh most DM sufferer in the world.[1] These conditions are very worrying that DM will worsen the incidence of other diseases,[2] especially in the current COVID pandemic.[3] DM can be grouped into four categories, type 1 diabetes mellitus (T1D) which is a failure of insulin production and is usually congenital, type 2 diabetes mellitus (T2D) is an event that is more likely to be experienced by adults and is caused by genetic disorders and environmental factors. Gestational diabetes (GD) is usually owned by women who are pregnant, and the last is diabetes with other symptoms that may be caused by infectious disease syndrome of the pancreas or the use of drug substances in the case of HIV AIDS therapy or after organ transplantation.[4,5]

T2D is characterized by increased levels of glucose in the blood due to the malfunctioning of cell receptors to enter insulin, several studies show that it followed by decreasing insulin production.[6] T2D incidence can change the stability of body fluids.[7,8] The complexity of T2D makes it difficult to control.[9] T2D is caused by genetic factors and environmental factors. Genetic factors are related to factors that cannot be changed and are inherited from both parents, while environmental factors are the factors that sufferers can manipulate, including changing social habits, especially due to lack of physical activity and excessive food consumption.[5,10]

Analysis of the relationship between the effects of genetic mutations with T2D is widely carried out. More than 120 genes are involved and some are closely related.[11,12,13,6,14,15] However, type 2 DM is still difficult to control due to other factors that have a tendency such as obesity,[16] ethnicity,[17] and lifestyle changes.[18]

Previous analyzes regarding the identification of polymorphisms showed different polymorphisms between type 2 DM and non-DM patients.[19] PCR-RAPD has advantages such as simple, easy, inexpensive, does not require target gene analysis, and can analyze the whole genome. The purpose of this study was to determine specifically as a marker of the possibility of a person suffering from type 2 diabetes or not using primers that produce specific polymorphisms.

2. MATERIAL AND METHOD

This research method was cross-sectional with an exploratory descriptive. Sampling had approved by the ethical committee of dentistry, Faculty of Medicine, Airlangga University, Surabaya with number: 091 / HRECC.FODM / III / 2020. A total of 60 people consisting of 30 positive T2D and 30 negative T2D were used in this study. Sampling through macro sampling as much as 3 cc is stored in the EDTA tube. Sampling was done by purposive sampling. The inclusion criterion for a positive sample was had diagnosed by a doctor or glucose test showed more than 200 mg/dl (random blood test). A negative sample is a subject that has less than 180 mg/dl in the glucose test. An interview was also given whether he had a family history of DM or not. If the sample had a family history of DM, the sample was canceled.

DNA isolation was carried out using the standard procedure of the GeneAid DNA Isolation kit for blood. The pure DNA was then analyzed quantitatively using a Thermo Evolution 201 spectrophotometer. PCR-RAPD was done using Bio-rad T100 thermocycler with 15 µL total reaction (DNA Template 2 µL, Primer A10 2 µL (5'-ACCCGGTCAC-3 '), PCR Mix 5 µL, ddH2O 6 µL). The reaction was Pre-denaturation 94˚C at 5 minutes, Denaturation 94˚C at 1 minute, Annealing 36˚C at 1 minute, Extension 72˚C at 2 minutes, Cycle 45 cycles, Final Extension 72 ˚C at 10 minutes. The band PCR-RAPD results were visualized with 2% gel agarose and were analyzed using Chi-square (cl: 95%) were done with SPSS ver.16.

3. RESULTS AND DISCUSSION

A total of 60 people from different individuals (30 positives for T2D & 30 as control) were used to look for molecular markers on T2D. The primer used is D20 (5'-ACCCGGTCAC-3'). This study strengthens previous research that has shown polymorphism in T2D samples.[19] Multiplication of samples and statistical analysis were carried out as well as looking for polymorphism characteristics from Sidoarjo, Indonesia.

PCR-RAPD produced 16 bands ranges from 174 bp to 3677 bp in length. Three bands were monomorphic (478 bp, 944 bp, & 2109 bp) and the others were polymorphic (Table 1). It was found that the band at 576 bp was significantly different between controls and T2D (p-value: 0.001). The number of bands found shows not the same number as the previous study.[19] However, the lengths were found to be different. The differences in polymorphisms previously were found around 1500 bp.

Confirmation using statistical tests is needed for this study because Zahid[19] studies used a relatively small sample. With the use of more waste, the reliability and accuracy of the research can be accepted. However, RAPD requires a higher reliability test than other methods. The reason for the band appearance is one of the considerations for using the

molecular marker. The clear of band target appearance above the monomorphic material is 476 bp (Figure 1). This will make it easier to see. Based on the percentage, the value of the band that appears in the positive sample is still low, which shows that 40% or 12 people with T2D sufferers have this band. This value is still very small because the marker may use at least 80% of the sample to have this band.

Previous research shows differences in bands than this study. Six bands show the highest polymorphism where are not found in control samples but found in positive samples. The highest polymorphism is at around 1500 bp in length. However, it was found to be far from the previous. The difference in results is possible because the specifications of each sample origin are different.[17]

The use of the 576 bp must be supported by other research that is mutually reinforcing. To strengthen the genes that are specifically involved, it needs to be confirmed. The use of the 576 bp must be supported by other research. To strengthen the genes that are specifically involved, it needs to be confirmed. The number of presence of 576 bp bands found was 40%. The use of primers that produce different bands can give the best results.

PCR-RAPD analysis was impossible to identify genes specifically. PCR-RAPD method also produces many bands and most of its unnecessary. PCR-RAPD analysis is powerful when several primers that produce statistic significantly different polymorphisms are used together to detect the possibility of a person being exposed to T2D or not. it also can be used as a marker for screening associated gene T2D in certain populations. Validation is recommended for this method. Although a person is genetically at risk, the risk for each person to experience T2D and complications is different.[19] The risk of T2D can be minimized by changing lifestyles.[18] The weakness of this study is the limited information on the personal object detected, such as weight, another disease status, age, etc. so that a deeper discussion cannot be done.

CONCLUSION

The polymorphism analysis comparing T2D sample and controls found significant differences in the 576 bp band (p-value 0.001) and can be used as candidates for molecular markers for these genetic disorders. The use of PCR-RAPD to molecular markers requires support for the other primers that produce specific bands.

DISCLOSURE STATEMENT

The authors declare that they have no conflict of interest.

ACKNOWLEDGEMENT

Our thanks go to DRPM UMSIDA who funded this research by internal grant 2020 and the molecular biology team who worked hard on the research. Molecular biology laboratory which has provided the facilities.

FUNDING INFORMATION

Internal grant.

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SHORT BIOGRAPHY

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Tabel 1. Frequency of polymorphic bands in diabetics and control subjects based on D20 Primer

No	Polymorphic band size (bp)	Number of Sample		% Sample		P-Value
		Control	DMT2	Control	DMT2	
1	174	6	2	20.0	6.7	0.129
2	255	7	10	23.3	33.3	0.390
3	312	9	7	30.0	23.3	0.559
4	353	28	29	93.3	96.7	0.554
5	405	26	27	86.7	90.0	0.688
6	476	28	28	93.3	93.3	1
7	576	1	12	3.3	40.0	0.001
8	623	11	12	36.7	40.0	0.791
9	716	27	28	90.0	93.3	0.640
10	784	26	29	86.7	96.7	0.161
11	944	29	29	96.7	96.7	1
12	1163	27	29	90.0	96.7	0.301
13	1519	6	9	20.0	30.0	0.371
14	2109	30	30	100.0	100.0	1
15	2381	10	15	33.3	50.0	0.190
16	3677	2	4	6.7	13.3	0.389

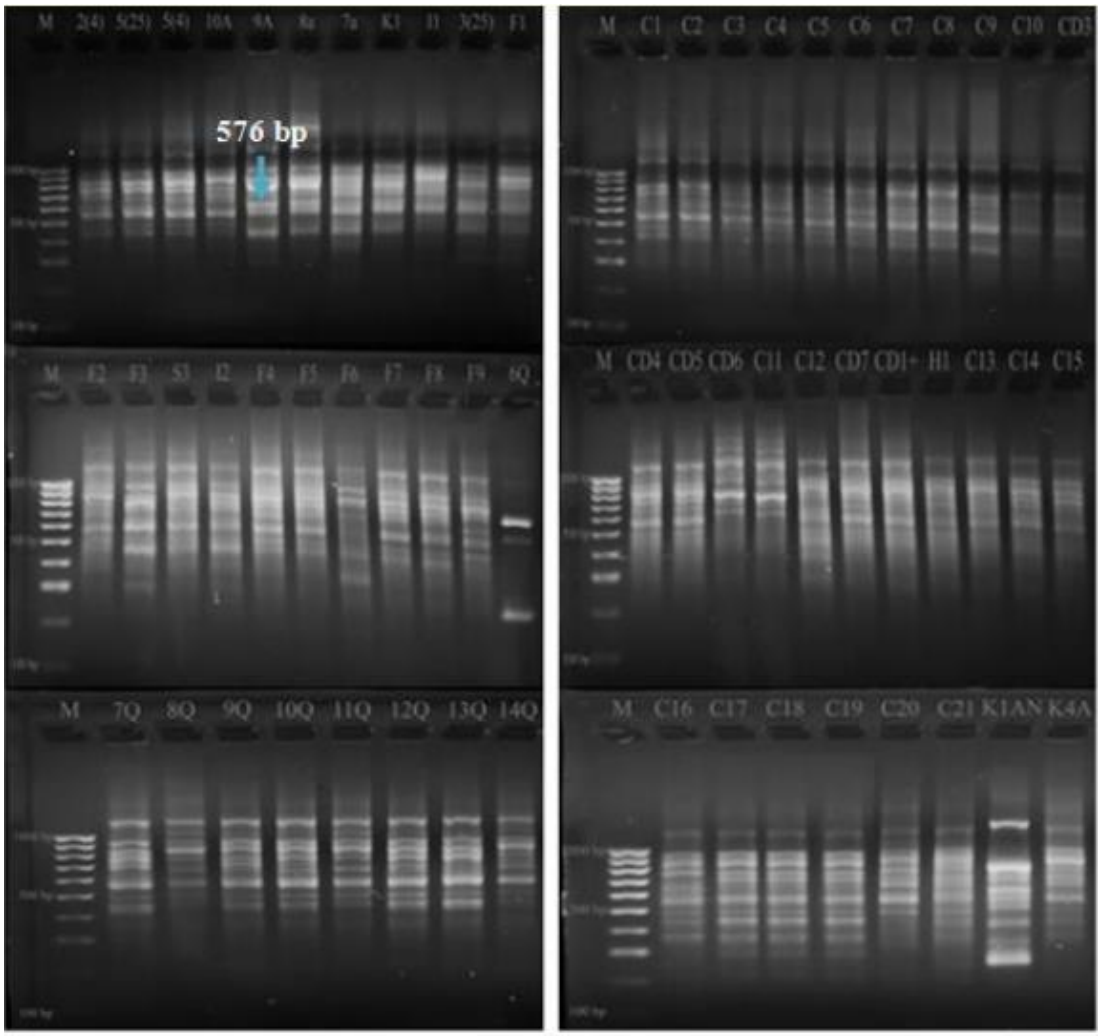

Figure 1. RAPD amplification products generated by primer D20. electrophoresis in 1.2% agarose gel, M is 100 bp DNA ladder

A: positive T2D, B: Control